

determining algorithm described herein. The following sequences were selected.

- I.. N-SQDEVREN-C
- II. N-ELSRESREGR-C
- III. N-RVLEEEENK-C
- IV. N-QKCDSVEE-C
- V. N-EETGINRERKVE-C
- VI. N-EPEIELPREPRNEE-C

B. Materials and methods

- i. Hep G2 cells were seeded into six-well dishes at 30,000 cells/well in MEM-supplemented media with 10% FBS. Cells were grown to near confluency and exposed to
  - I. media alone
  - II. Mevastatin 5 $\mu$ Mol (Cholesterol-lowering Statin drug) and,
  - III. HMG CoA Reductase peptides at 1, 10 and 100  $\mu$ Mol.
- ii. All treatments were carried out in triplicate. After 24 hrs the media was removed and saved.
- iii. The cells were washed with 1 ml of PBS and combined with the media. The cells were harvested with 1 ml of 0.1N NaOH.
- iv. Samples, both media and cells, were saponified with 0.5 ml 50% KOH, 3 ml ethanol, separately at 80°C for 2 hrs.
- v. After cooling, the samples were extracted twice with petroleum ether and washed with NaOH.
- vi. The samples were dried under nitrogen and suspended in 200  $\mu$ l ethanol.
- vii. 50  $\mu$ l of this suspension was used for determination of cholesterol. Cholesterol was determined in the samples and standards using colorimetric assay (Sigma Kit).
- viii. Protein was determined by Lowry's method and the results were expressed as  $\mu$ gm cholesterol/mg protein.

C. Results

i. The following results were obtained:

<u>Peptide</u>	<u>Treatment</u>	<u>Cholesterol</u> <u>Content</u>		<u>Total</u> <u>Cholesterol</u> ( $\mu$ gm/mg protein)	<u>Cholesterol</u> <u>Lowering in</u> <u>% of Control</u>
		Cell	Media		
	Control	152.1 $\pm$ 39.0	66.5 $\pm$ 6.5	218.6 $\pm$ 35.0	-
	Mevastatin 5 $\mu$ M	124.4 $\pm$ 4.4	57.5 $\pm$ 5.2	182.0 $\pm$ 7.0	18.0
1	1 $\mu$ M	92.5 $\pm$ 4.7	65.2 $\pm$ 4.0	158.7 $\pm$ 5.6	28.0
	10 $\mu$ M	88.6 $\pm$ 1.6	60.6 $\pm$ 0.5	149.1 $\pm$ 2.0	30.0
	100 $\mu$ M	94.0 $\pm$ 4.0	61.1 $\pm$ 2.4	155.3 $\pm$ 6.4	30.0
2	1 $\mu$ M	94.0 $\pm$ 8.3	64.3 $\pm$ 4.2	158.3 $\pm$ 6.3	29.0
	10 $\mu$ M	85.0 $\pm$ 8.4	63.7 $\pm$ 9.5	148.6 $\pm$ 11.8	33.0
	100 $\mu$ M	86.0 $\pm$ 4.7	59.0 $\pm$ 6.0	146.9 $\pm$ 8.8	34.0
3	1 $\mu$ M	137.3 $\pm$ 4.5	64.0 $\pm$ 2.7	201.3 $\pm$ 3.3	10.0
	10 $\mu$ M	135.0 $\pm$ 4.5	65.5 $\pm$ 4.7	200.7 $\pm$ 9.0	10.0
	100 $\mu$ M	135.0 $\pm$ 7.0	65.5 $\pm$ 1.4	200.5 $\pm$ 5.4	10.0

Get  
Q2

<u>Peptide</u>	<u>Treatment</u>	<u>Cholesterol</u> <u>Content</u>		<u>Total</u> <u>Cholesterol</u> ( $\mu$ gm/mg protein)	<u>Cholesterol</u> <u>Lowering in</u> <u>% of Control</u>
		Cell	Media		
	Control	151.0 $\pm$ 7.8	50.8 $\pm$ 2.5	202.0 $\pm$ 5.3	-
	Mevastatin 5 $\mu$ M	125.0 $\pm$ 4.6	37.5 $\pm$ 3.0	162.6 $\pm$ 7.6	19.0
4	1 $\mu$ M	134.7 $\pm$ 3.2	50.4 $\pm$ 2.5	184.3 $\pm$ 5.5	8.0
	10 $\mu$ M	126.1 $\pm$ 4.2	49.0 $\pm$ 1.0	175.2 $\pm$ 3.8	10.0
	100 $\mu$ M	127.7 $\pm$ 9.3	53.8 $\pm$ 0.5	181.7 $\pm$ 9.3	9.0
5	1 $\mu$ M	140.7 $\pm$ 8.6	56.8 $\pm$ 1.0	197.0 $\pm$ 9.2	1.5
	10 $\mu$ M	149.0 $\pm$ 4.4	50.7 $\pm$ 1.0	200.0 $\pm$ 5.0	0.0
	100 $\mu$ M	161.7 $\pm$ 23.5	48.0 $\pm$ 1.6	210.0 $\pm$ 25.0	0.0
6	1 $\mu$ M	144.3 $\pm$ 7.5	47.4 $\pm$ 0.5	192.0 $\pm$ 8.0	4.0
	10 $\mu$ M	136.0 $\pm$ 12.2	48.0 $\pm$ 3.4	184.0 $\pm$ 8.4	8.0
	100 $\mu$ M	132.0 $\pm$ 4.4	50.4 $\pm$ 1.0	182.4 $\pm$ 4.8	9.0

#### REMARKS

Applicant respectfully submits that Example Number 6 does not constitute new matter. Rather, Example Number 6 provides experimental data in support of the presently pending claims.

A Final Office Action relating to the U.S. application serial number 09/232,186 was issued on August 8, 2000. Applicant filed a Notice of Appeal on February 8, 2001, therefore an Appeal Brief was due on April 8, 2001. Applicant hereby requests a THREE-MONTH extension time in lieu of an Appeal Brief; i.e., the filing of this continuation.

The Commissioner is hereby authorized to charge payment of the 37 C.F.R. § 1.136(a) extension fee to